Outcalt K. W. (1991), *Seed Sci. & Technol.*, **19**, 511–517

Stratification increases germination of Ocala sand pine seed in dry soil

KENNETH W. OUTCALT

Southeastern Forest Experiment Station, Post Office Box 70, Olustee, FL 32072, USA

(Accepted February 1991)

Summary

Stratifying, i.e., soaking followed by chilling, Ocala sand pine seed before sowing significantly increased both speed and completeness of laboratory germination, with an aerated soak the most beneficial. Even at 3% sand moisture content, 40% of seed given an aerated soak followed by moist chilling treatment had germinated after eight days. Neither soaking alone nor seed coat removal increased germination rates, while presoaked and chilled seed retained the ability to germinate in dry sand even after redrying. In the field portion of the study five weeks after seed were sown 1.2 cm deep, germination was 29% and 5% for the soaked and control seed, respectively. Although Ocala sand pine seed is classed as non-dormant, it would benefit from a presowing stratification because it increases germination in dry soils.

Introduction

Ocala sand pine (*Pinus clausa* var, *clausa* D. B. Ward) is native to the infertile and droughty marine deposited sandhills of central Florida (U.S.A.). Precipitation across the area is abundant, 1320 mm per year, with more than half occurring from June to September (Burns and Hebb, 1972). Because of the low moisture holding capacity of the soil, drought conditions can develop within two weeks of a heavy rainfall. Artificial regeneration is accomplished by direct seeding from November to February, when temperatures and soil moisture are most favorable. But even during this period, stressful dry conditions often occur, delaying germination of non-dormant seed (Barnett, 1973) and increasing predation losses – the major obstacle to successful establishment of tree species by direct seeding (Abbott, 1974; Cooper, Schopmeyer and McGregor, 1959; Derr and Mann, 1971; Williston and Balmer, 1977).

Priming, the osmotic conditioning of seed (Heydecker, Higgins and Gulliver, 1973), has been shown to improve both rate and completeness of germination in a number of agricultural species (Bradford, 1986). Osmotic conditioning with polyethylene glycol gives the same benefits when applied to seed of slash pine (*Pinus elliottii* Engelm.) (Haridi, 1985). There also is evidence that presowing treatments such as priming increase the ability of seed to germinate under stressful environmental conditions (Bradford, 1986; Tanaka, Kleyn and Harper, 1986). The objective of this study was to determine if a presowing treatment of Ocala sand pine seed would speed germination

under stressful conditions, and if it would increase the number of seedlings under field conditions.

Materials and Methods

All seed used in the study were from four seedlots used for regeneration on the Ocala National Forest, Florida, during the 1986 season. Seed were originally obtained from collections of new cones from the fall 1985 timber sale areas scattered about the forest. After collection, cones were shipped to the USDA Forest Service's Ashe nursery in Mississippi where they were opened by dipping for 5 to 10 seconds in boiling water followed by kiln drying at 45°C for 24 to 48 hours. After seed were extracted from open cones by shaking, they were dewinged, cleaned, and shipped back to the Ocala National Forest where they were kept in cold storage until used in this study commencing during the fall, 1987.

A randomized block, split plot design was used for the first phase of the laboratory, as well as the field, portion of this study. In the laboratory, sand moisture content was the main plot, and presowing seed treatment was the subplot variable. Treatment one consisted of soaking seed in distilled water for 24 hours with constant light at 25°C, draining excess water, and moist chilling for 28 days in sealed containers at 4°C, followed by surface drying. Equal volumes of water and seed, where seed volume included the seed and the air space between seed, was used for the soaking. The second treatment was the same except that soaking water was aerated by bubbling compressed air into the bottom of the container at a rate of 6 liters per minute for each liter of water, causing the water to mix in a manner comparable to water at a moderate boil. For the third (control) treatment, seed were kept dry, but were exposed to the same light and temperature regimes as for treatments one and two. Seed used for all treatments were from a mixture of the four seedlots.

At the end of the treatment period measured amounts of distilled water were added to 200 g of sand, which had been oven-dried (105°C) and then cooled, to give sand moisture levels of 3, 5 and 7% on a dry weight basis. This is equivalent to moisture potentials of 90, 60 and 40-kPa respectively, as determined previously using resistance blocks. After thorough mixing, sand was lightly packed in plastic germination boxes and a small plastic marker was placed across the center of each box. Seed, 50 control on one side and 50 presoaked on the other in five rows of 10 each, were sown in each box by placing them with tweezers into holes made with a pencil point to a depth of 0.6 cm. After sowing, seed were covered by lightly brushing sand into the holes with the tweezers. When sowing was completed, lids were placed on the boxes which were stored in a germination room at 25°C with constant light.

A randomized block design with three blocks, four treatments, and the four seedlots was used in the second phase of the laboratory portion of the study. The control and 24-hour aerated soak followed by moist chilling (described above) were two of the treatments employed. A third treatment was a 24-hour aerated soak followed by surface drying, and treatment four was as three, but with the seedcoat removed after

soaking and surface drying. Treatments were applied so all seed were ready to sow at the same time. After treatment, seed were placed on germination paper in plastic germination boxes, wet with distilled water, and placed in the germination room.

In the third phase of the laboratory portion of the study, seed from the four seedlots were given the 24-hour aerated soak treatment followed by 28 days of moist chilling. Following surface drying at 28 days, three replications of 150 seed from each seedlot were placed in plastic boxes in sand at 2% moisture content. Boxes were stored in the germination room at 25 °C with constant light. On days 0, 1, 5, 8, 14 and 21, ten seed were removed from each box and weighed. After oven drying for 24 hours at 103 °C, seed samples were cooled in a dessicator and reweighed. At the end of 21 days the sand was screened and the remaining seed recovered. Fifty of these recovered seed from each box were then sown, along with 50 untreated control seed from the same lot, in sand at 3% moisture content using a randomized block split plot design.

In the field portion of the study, planting depth (0.6 or 1.2 cm) was the main effect and presoaking seed treatment was the subplot effect. Seed treatment two, the aerated soak followed by moist chilling, and three, the control, as explained in the first phase of the laboratory study, were compared. Three sites were selected at random for seeding in November 1988 from sites on the Lake George district of the Ocala National Forest, which had been scarified three weeks earlier using the Bracke® scarifier-seeder (Damme, 1988). Three seeding areas, each in the center of a Bracke spot, were randomly selected on each site. At each planting site 100 holes were made by pressing 4 mm diameter hollow tubes, attached to a 30 × 30 cm board in a 10 × 10 configuration and protruding the two desired depths, into bare mineral soil. Control seed were placed with tweezers into 50 holes, 25 at each of the two depths, and aerated, presoaked seed were placed in the other 50 holes, and all were covered with soil.

Number of normal germinants (ISTA, 1985) was recorded daily for 28 days in the laboratory portion of the study and used to calculate germination capacity, germination rate (Edwards and El-Kassaby, 1988), and germination value (Czabator, 1962). Germination capacity is the percentage of seed which germinated before the study's end; germination rate the number of days to reach 50% germination; and germination value specifies the mean daily germination multiplied by the peak daily value of germination. Germination capacity after five weeks was used to evaluate the field phase of the study. Following arc-sin transformation, results were analyzed by analysis of variance. The Least Significant Difference method was used to compare treatment means.

Results

During the first phase of the laboratory study, presowing seed treatments more than tripled total germination (table 1). Germination rate was also faster for treated seed, with none of the control treatments reaching 50% germination, even after 28 days. Aeration during the soaking part of the presowing treatment significantly benefited seed by increasing both completeness and speed of germination.

Table 1. Total germination, G 50 (days to 50% germination), and germination value (Czabator, 1962) of Ocala sand pine seed after different presowing treatments.

Seed treatment	Germination (percent)	G 50 (days)	Germination value
Control	15 a*	ND**	0.2 a
Nonaerated soak and 28 day moist chilling	55 b	16 b	3.5 b
Aerated soak and 28 day moist chilling	72 c	10 a	7.4 c

* Within each column, means followed by the same letter are not significantly different at the 0.05 level. Means are averages of nine numbers from three sand moisture levels for each of three blocks.
** Controls did not reach 50% germination.

At all sand moisture levels, the aerated soak treatment had the quickest and highest total germination, while the control treatment always performed the poorest (figure 1). There was no difference in germination at 5 and 7% sand moisture content. At a moisture content of 3%, however, germination was reduced for all treatments, especially in the control.

There were no significant differences between seedlots in speed or completeness of germination in the second or third phases of the laboratory study. Removal of the seedcoat prior to sowing reduced total germination (table 2). Moist chilling for 28 days following the aerated soak significantly increased the germination rate, while an aerated soak alone or soaking and seedcoat removal had no effect on germination rate.

After an aerated soak and 28 days of chilling, seed in the third phase of the laboratory study had an average moisture content of 27% on a dry weight basis. There was no change in seed moisture during the first day these seed were placed in sand with 2% moisture content (sand moisture potential 200-kPa). After five days in the dry sand, seed moisture levels had fallen to 19%; by day eight moisture levels had fallen to 10%; and after 14 days seed had stabilized at 8% moisture. Germination was 39%

Table 2. Effect of presowing seed treatments on total germination and G 50 (days to reach 50% germination) of Ocala sand pine seed.

Seed treatment	Germination (percent)	G 50 (days)
Control	63 b*	11 a
Aerated soak	80 a	11 a
Aerated soak and seedcoat removed	40 c	12 a
Aerated soak and 28 day chilling	74 ab	6 b

* Within each column, means followed by the same letter are not significantly different at the 0.05 level. Means are average of 12 numbers, i.e., four seedlots in each of three replications.

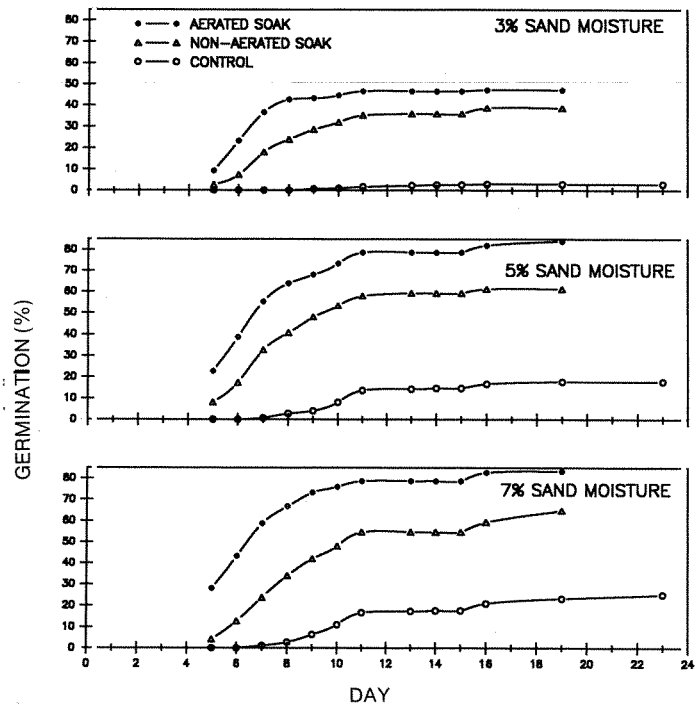

Figure 1. Effects of sand moisture content on the germination of Ocala sand pine seed given different presowing treatments.

when seed with this 8% moisture was then sown in sand with 3% moisture. None of the control seed germinated after sowing in sand with moisture content of 3%.

In the field portion of the study, depth of sowing, seed treatment, and their interaction all significantly affected germination after five weeks (table 3). Seed sown 1.2 cm deep germinated better than that sown 0.6 cm. Presowing treatment of seed by an aerated soak followed by chilling also increased germination. The greatest average

Table 3. Effect of planting depth and presowing treatment on the germination of Ocala sand pine seed after five weeks in the field.

Planting depth (cm)	Germination %	
	control	aerated soak and moist chilled
0.6	0 a*	3 ab
1.2	5 b	29 c

* Means followed by the same letter are not significantly different at the 0.05 level. Means are average of 12 numbers, i.e., four seedlots in each of three replications.

germination occurred when the aerated soak treatment followed by chilling was combined with deeper sowing, with an increase of about six times over either treatment used alone.

Discussion

The 24-hour water soak followed by 21 to 28 days of moist chilling is a common system of stratification for southern pine seed. It has also been used extensively to break dormancy of western conifer seed, where it has been referred to as 'naked stratification' (Tanaka, 1984). It has not been used routinely on Ocala sand pine seed as this seed is classed as non-dormant since it will germinate relatively quickly and completely under favorable conditions. Dormancy, however, is a relative term, since the seed is in a resting state where germination cannot occur until certain physiological processes have taken place. Alvarado and Bradford, (1988) and Savino, Haigh and DeLeo, (1979) have suggested that the activation of physiological processes during presowing seed treatments are the cause of an increased germination rate, and this appears to be the case for Ocala sand pine. The lack of a response to soaking without moist chilling in the second phase of the laboratory study indicates that the increased rate of germination was not due to the removal of some inhibitory substance. Barnett, (1970) also found no evidence of any germination inhibitors in Ocala sand pine seed.

For the sand used in the laboratory portion of the study, permanent wilting point and field capacity equate to about $^1/_2$% and 15% moisture, respectively. No water was added to the germination boxes during the 28 days of the study, and moisture loss did occur because the lids did not seal completely. Even at a beginning moisture content of only 3%, the seed given the aerated presoak followed by moist chilling treatment reached 40% germination after just eight days, while germination was only 3% for the control seed. The ability to germinate in relatively dry conditions was not due to moisture stored in the seed, since treated seed retained this ability in the third phase of the laboratory test even after they had redried to a moisture content below the 10% level of control seed. Bradford, (1986) indicates there is an osmotic adjustment during this type of presowing treatment that results from the accumulation of solutes which lower seed water potential. This probably explains the greater germination rate and capacity for the presoaked seed under dry conditions. Thus, the increased germination rate in moist and dry sand seems due to metabolic processes which occur during the presoaking and moist chilling treatment. Traditionally, these processes have not been associated with dormancy in Ocala sand pine seed, but this view may need to be revised. It is clear that even seed considered to be non-dormant can benefit from stratification since the ability of seed to germinate under stressful conditions can be improved.

The aerated water soak followed by chilling also increased germination in the field part of the study. The gain was of little practical significance however, unless seed were sown 1.2 cm deep. This could partly be due to greater seed predation – since predators should be more successful at finding seed which are located closer to the

surface. The higher moisture content deeper in the soil is more likely responsible for the difference however, since the upper 0.6 cm of soil dries rapidly from evaporation, while beyond this point soil moisture seldom falls below the wilting point unless it is being displaced by plant roots (Burns and Hebb, 1972).

The results of this study show that when seed are given an aerated soak and are sown at the proper depth, even during dry periods, most areas should be successfully regenerated.

References

Abbott, H. G. (1974). Direct seeding in the United States. In *Proceedings of Direct Seeding Symposium,* (ed. J. Cayford), Canadian Forestry Service Publication No. 1339, pp. 1–10. Canadian Forestry Service, Ottawa, Can.

Alvarado, A. D. and Bradford, K. J. (1988). Priming and storage of tomato (*Lycopersicon lycopersicum*) seeds. I. Effects of storage temperature on germination rate and viability. *Seed Science and Technology,* **16**, 601–612.

Barnett, J. P. (1970). Germination inhibitors unimportant in dormancy of southern pine seeds. *USDA Forest Service Research Note, SO 112,* pp. 1–4. USDA Forest Service, New Orleans, LA.

Barnett, J. P. (1973). Sand pine: cone and seed traits. In *Proceedings of Sand Pine Symposium.* General Technical Report SE-2, pp. 55–66. USDA Forest Service, Asheville, NC.

Bradford, K. J. (1986). Manipulation of seed water relations via osmotic priming to improve germination under stress conditions. *HortScience,* **21**, 1105–1112.

Burns, R. M. and Hebb, E. A. (1972). Site preparation and reforestation of droughty, acid sands. *USDA Forest Service Agriculture Handbook*, **426**, pp. 1–61. USDA Forest Service, Washington, D.C.

Cooper, R. W., Schopmeyer, C. S. and McGregor, W. H. D. (1959). Sand pine regeneration on the Ocala National Forest. *Production Research Report No. 30*, pp. 1–37. USDA Forest Service, Washington, D.C.

Czabator, F. J. (1962). Germination value: An index combining speed and completeness of pine seed germination. *Forest Science*, **8**, 386–396.

Damme, L. van. (1988). Sowing method and seed treatment effects on jack pine direct seeding. *Northern Journal of Applied Forestry*, **5**, 237–240.

Derr, H. J. and Mann, W. F. (1971). Direct-seeding pines in the south. USDA Forest Service *Agricultural Handbook No.* **391**, pp. 1–68. USDA Forest Service, Washington, D.C.

Edwards, D. G. W. and El-Kassaby, Y. A. (1988). Effect of flowering phenology, date of cone collection, cone-storage treatment and seed pretreatment on yield and germination of seeds from a Douglas-fir seed orchard. *Forest Ecology and Management*, **25**, 17–29.

Haridi, M. B. (1985). Effect of osmotic priming with polyethylene glycol on germination of *Pinus elliottii* seeds. *Seed Science and Technology*, **13**, 669–674.

Heydecker, W., Higgins, J. and Gulliver, R. L. (1973). Accelerated germination by osmotic seed treatment. *Nature*, London, **246**, 42–44.

International Seed Testing Association (1985). International rules for seed testing. *Seed Science and Technology*, **13**, 299–513.

Savino, G., Haigh, P. M. and DeLeo, P. (1979). Effects of pre-soaking upon seed vigour and viability during storage. *Seed Science and Technology*, **7**, 57–64.

Tanaka, Y. (1984). Assuring seed quality for seedling production: Cone collection and seed processing, testing, storage and stratification. In *Forest Nursery Manual: Production of Bareroot Seedlings* (ed. M. Duryea and T. Landis), pp. 27-39. Martinus Nijhoff/Dr. W. Junk, The Hague.

Tanaka, Y., Kleyn, N. J. and Harper, L. M. (1986). Seed stratification of Engelmann spruce and lodgepole pine: the effect of stratification duration and timing of surface-drying. *Forestry Chronicle*, **62**, 147–151.

Williston, H. L. and Balmer, W. E. (1977). Direct seedling of southern pines – A regeneration alternative. *Forest Management Bulletin*, pp. 1–6. USDA Forest Service, Atlanta, GA.